CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-498

Statistical Review(s)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF BIOSTATISTICS

Statistical Review and Evaluation

CLINICAL STUDIES

NDA:	— / 21-498
Name of drug:	— nitazoxanide 100mg / 5Ml oral suspension.
	Drug Regimen: b.i.d. Treatment Duration: 3 days
Applicant:	Romark Laboratories, L.C.
Indication:	Treatment of Diarrhea caused by *Cryptosporidium parvum*, *Giardia lamblia* —
Clinical Division	Division of Special Pathogen and Immunologic Drug Products (DSPIDP), HFD-590
Project manager:	Kristen Miller (DSPIDP, HFD-590)
Clinical reviewer:	Rosemary Johann-Liang, M.D. (DSPIDP, HFD-590)
Dates:	Submission 5/29/02; 45 day meeting 6/21/02; user fee (6 months) 11/29/02.
Documents reviewed:	Volumes 1.1 and 1.32-1.34, 1.38-1.39 and electronic submission found under \\CDSESUB1 — 000\2001-05-29
Statistical reviewer:	Jyoti Zalkikar, Ph.D. (DBIII, HFD-725)
Statistics team leader:	Karen Higgins, Sc.D. (DBIII, HFD-725)
Biometrics division director:	Mohammad Huque, Ph.D. (DBIII, HFD-725)

Keywords: Clinical Studies, NDA review, Single-Center Studies, Superiority Trials, Non-inferiority Trials, Kappa coefficient

1 EXECUTIVE SUMMARY OF STATISTICAL FINDINGS

1.1 CONCLUSIONS AND RECOMMENDATIONS

The submissions, ——— NDA 21-498, by Romark Laboratories include five adequate and well-controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide (NTZ) in treating diarrhea caused by *C. parvum* and *G. lamblia*. **All five studies were conducted at single centers in endemic areas, namely Egypt, Zambia and Peru.** Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator. The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS. Both clinical and parasitological endpoints were used to evaluate efficacy. The responses were measured approximately 4 days after the end of treatment. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'. Efficacy data was analyzed using intent-to treat (ITT) population generally defined as all patients who took at least one dose of the treatment.

Single Center Studies

All five studies in this application were designed as single center, single race studies conducted outside of Unites States. This fact seriously compromises the generalizability of the results of these studies to the population in Unites States, and applicability of these results to future non-inferiority studies where NTZ, if approved, may be used as an active comparator. The data from these studies could not be used to assess the effects of important covariates such as race, age group, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

It is strongly recommended that any future studies in the development of NTZ be designed and conducted as adequate, well-controlled, **multi-center** studies.

C. Parvum

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum* : ——— ——— . However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center studies in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*, [redacted]

Giardia Lamblia

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Giardia Lamblia* [redacted]

It is recommended that any future studies be conducted as placebo controlled, multi-center studies in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lamblia,* [redacted]

1.2 OVERVIEW OF CLINICAL PROGRAM AND STUDIES REVIEWED

Nitazoxanide was originally synthesized in the early 1970s, but was not fully developed at that time. Romark Laboratories re-initiated the development of nitazoxanide in the 1990s for treating infections caused by a broad spectrum of parasites that infect intestinal tracts of humans including *Cryptosporidium parvum* infections. The current submissions, [redacted] NDA 21-498, include five adequate and well-controlled clinical studied that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide in treating diarrhea caused by *C. parvum* and *G. lamblia*. Both of these diseases are considered orphan diseases in the United States.

All five studies were conducted at single centers in endemic areas, namely Egypt, Zambia and Peru. Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator.

The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS.

All studies were designed similarly and used both clinical and parasitological endpoints to evaluate efficacy. Efficacy data was analyzed using an intent-to-treat (ITT) population generally defined as all patients who took at least one dose of the treatment.

The following table provides a brief overview of the five pivotal studies.

Table 1: Controlled Clinical Trials in NDAs —— 21-498

Study #	Center	Study Design	Treatement: dose	Sample size	Age range	Treatment Duration
Controlled Clinical Trials : Diarrhea Caused by C. Parvum						
RM-NTZ 98-002	Egypt	Randomized double-blind Placebo-controlled	NTZ: 100 mg bid	11	1-3	3 days
			200 mg bid	13	4-11	
			500 mg bid	25	12-62	
			Placebo:bid	50	1-62	3 days
RM02-3007	Zambia	Randomized double-blind Placebo-controlled	NTZ: 100 mg bid	25	1-3	3 days
			Placebo:bid	22	1-3	3 days
RM02-3008	Zambia	Randomized double-blind Placebo-controlled	NTZ: 100 mg bid	24	1-3	3 days
			200 mg bid	1	4-11	
			Placebo:bid	25	1-3	3 days
Controlled Clinical Trials : Diarrhea Caused by Giardia lamblia						
RM-NTZ	Egypt	Randomized double-blind Placebo-controlled	NTZ: 500 mg bid	47	12-65	3 days
			Placebo:bid	44	12-65	3 days
RM-NTZ 99-010	Peru	Randomized single-blind Active-controlled	NTZ: 100 mg bid	14	2-3	3 days
			200 mg bid	41	4-11	
			Metronidazole 125 mg bid	29	2-3	5 days
			250 mg bid	26	4-11	

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1.3 PRINCIPLE FINDINGS

C. Parvum

Three studies were conducted by the sponsor to demonstrate the efficacy of three-day regimen of NTZ in treating diarrhea caused by *C. Parvum.*

Adult Study Results

Adult HIV negative population (age ≥ 12 years) was studied in only one study, RM-NTZ-98-002, conducted at a single center in Egypt.

Single center, single race nature of this study seriously compromises the generalizability of the results of this study to the general population.

The difference between NTZ and placebo for the clinical endpoint ('well' response defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.') was not statistically significant (p-value=0.0423, one-sided). A statistically significant finding was reported for the parasitological endpoint ('Eradication' defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'). However, poor correlation between clinical and parasitological endpoints for the NTZ arm (kappa = -0.312) seriously compromises the meaningfulness of parasitological endpoint for this population.

Moreover, there were 8 patients (4- placebo, 4-NTZ) with mixed infections. When the analyses were carried out by excluding these patients (that is, for the adult population with only *C. Parvum* infection), the differences between NTZ and placebo were not statistically significant for both clinical and parasitological endpoints (p-values =.118). Subgroup analyses by gender, generally, did not show statistically significant difference between NTZ and Placebo for both clinical and parasitological endpoints.

Therefore, the data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum* [redacted] However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies Results

The population of HIV negative children (age 1-11 years) was studied in two randomized placebo controlled studies, one, RM-NTZ-98-002, conducted at a single center in Egypt and another, RM02-3007, conducted at a single center in Zambia.

Single center, single race nature of these studies seriously compromises the generalizability of the results of these studies to the general population. The data from these studies could not be used to assess the effects of important covariates such as race, malnourishment status, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

There were subtle differences in these two studies as follows. Ages of children in study RM-NTZ-98-002 ranged from 1-11 years, whereas the ages of children in study RM02-3007 ranged from 1 to less than 4 years, and all but two children in study RM02-3007 were malnourished. In study RM-NTZ-98-002, presence of *C. Parvum* at baseline was not re-confirmed, whereas in study RM02-3007, it was re-confirmed at baseline, and 3 male patients randomized to placebo group with no oocysts of *C. Parvum* at baseline were excluded from all efficacy analyses by the sponsor.

In both studies, the differences between NTZ and placebo were statistically significant (in favor of NTZ) in terms of clinical response (88% versus 38% in Study RM-NTZ-98-002 and 56% versus 23% in Study RM02-3007) and parasitological response (75% versus 20% in Study RM-NTZ-98-002 and 52% versus 14% in Study RM02-3007). However, sensitivity analyses and subgroup (based on age and gender)/special (only *C. Parvum* infection) population analyses did not, consistently, show statistically significant results. In both studies, kappa coefficients were positive for NTZ arm, but were not consistent across studies, age subgroups, gender subgroups and for the population of patients with only *C. Parvum* infection.

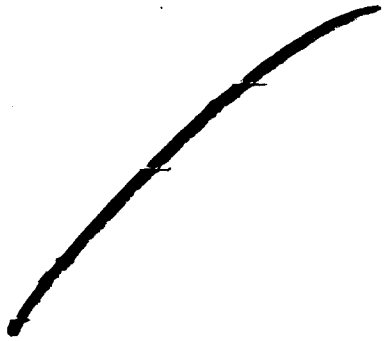
In study RM02-3007, there was statistically significant difference in the mortality rate within 7 days following enrollment between NTZ and Placebo (0% versus 18%, respectively).

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*,

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Another double-blind, placebo-controlled study was conducted in hospitalized, severely malnourished children of age 1-11 years with acquired immune deficiency syndrome (AIDS) in Zambia. In this study, a three-day course of nitazoxanide suspension (100 mg BID in children ages 12-47 months, 200 mg BID in children ages 4 through 11 years) did not produce clinical or parasitological response rates or mortality rates that were significantly different from the placebo control. Aside from their infection with HIV, the patients enrolled in this study were significantly more malnourished (mean weight-for-age z-scores: -5.5 compared to -3.5, $p<0.0001$); they reported a longer duration of diarrhea at time of enrollment (49 days compared to 20 days, $p< 0.01$); and they had lower CD4 cell counts

(mean: 620 cells/mm^3 compared to 1532 cells/mm^3, $p<.0001$) than those of HIV negative children enrolled in study RM02-3007.



It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Study Results

The population of HIV negative children (age 2-11years) with *Giardia Lamblia* infection was studied in only one active controlled study, RM-NTZ-99-010, conducted at a single center in Peru, where metronidazole was used as an active control.

Single center, single race nature of this study seriously compromises the generalizability of the results of this study to the general population.

In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole) , but not in terms of the parasitological response rates. The kappa coefficients for both NTZ and metronidazole arms were positive and comparable (0.276 for NTZ, 0.227 for Metronidazole). All subgroup/special protocol analyses in this study, such as ones based on gender, subgroup of patients with only *Giardia Lamblia*

infection, "per protocol" population, all showed results consistent with the Intent-to Treat population.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lamblia,* [redacted]

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

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2 INTRODUCTION

2.1 BACKGROUND

Nitazoxanide was originally synthesized in the early 1970s, but was not fully developed at that time. Romark Laboratories re-initiated the development of nitazoxanide (NTZ) in the 1990s for treating infections caused by a broad spectrum of parasites that infect intestinal tracts of humans including *Cryptosporidium parvum* infections.

In 1994, a program was initiated in 7 different sites in Egypt to evaluate efficacy and safety of NTZ. The focus was on patient's parasitological response to the treatment. The first dose regimen tested was 500 mg bid for three days in adults. After the safety of the drug had been established in approximately 200 adults, children were added to the study with the dose adjusted to 200 mg for age 4-11 years and to 100 mg for age 1-3 years. The 3-day regimen produced high eradication rates for a broad spectrum of protozoa and helminths.

To confirm the efficacy of the 3-day course of NTZ in treating intestinal parasitic infections, another uncontrolled study was conducted in adults and children in Mexico with mixed infections by protozoa and helminths (at least 3 parasites per subject). Again the focus was patients' parasitological response to treatment. The findings of this study were consistent with those of the Egyptian study.

The current submissions, [redacted] NDA 21-498, include five adequate and well-controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide in treating diarrhea caused by *C. parvum* and *G. lamblia*. Both of these diseases are considered orphan diseases in the United States. Currently there are no approved drug products labeled for the treatment of diarrhea caused by *C. Parvum*.

All of the five studies were designed based on guidelines published in the literature(1) for the evaluation of new drugs for treating diarrhea caused by Giardia lamblia. All patients selected for inclusion in these studies were symptomatic with diarrhea and were diagnosed with the enteric protozoan pathogen under study by stool examination. The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS.

Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator.

Both clinical and parasitological endpoints were used to evaluate efficacy. The responses were measured approximately 4 days after the end of treatment. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed

stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

Efficacy data was analyzed using intent-to treat (ITT) population in all the studies. However, in studies [redacted] and RM-NTZ-98-002, the protocols allowed the diagnosis within 7 days prior to enrollment and all patients enrolled had diarrhea at the time of enrollment, but the parasitological diagnosis was not reconfirmed at baseline. In the other three studies, the diagnosis was re-confirmed at baseline, and patients for whom the diagnosis was not reconfirmed were excluded from the ITT population and therefore from the efficacy analyses.

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2.2 DATA ANALYZED AND SOURCES

In order to recruit a sufficient number of patients to evaluate the efficacy of NTZ, all five pivotal studies were conducted in endemic areas, namely, Egypt, Peru and Zambia. All five studies were single center studies. The sponsor stated that the following efforts were made to ensure the quality of the studies:

1> The sites selected were willing to work with a team of foreign investigators and had patient availability, personnel and facilities that would allow completion of a study over a 12-month period.
2> Staff on site included a physician qualified in tropical medicine and one clinical research associate.
3> Additional personnel including physicians, parasitologists and clinical research associates were sent to the sites for short time periods to conduct study monitoring and quality assurance of parasitological examinations.
4> Romark's Quality Assurance staff audited the studies to assure compliance with the protocols.

The data sets for all five studies were submitted electronically at the following location:

\\CDSESUB1_____N_000\2001-05-29\CRT\Datasets

This reviewer found the efficacy data sets to be well organized and of good quality. Also this reviewer did not find notable discrepancies between the results given in the text of the sponsor's study report and those obtained using the submitted data sets.

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3 STATISTICAL EVALUATION OF EVIDENCE ON EFFICACY / SAFETY

3.1 DIARRHEA CAUSED BY *C. PARVUM*

3.1.1 STUDY DESIGN

The sponsor conducted three randomized, double-blind, placebo-controlled studies to evaluate efficacy of NTZ in the treatment of diarrhea caused by *C. Parvum*. Dosing and study design information is given in Table 1. Study RM-NTZ-98-002 was conducted in adults and children at a single center in Egypt, study RM02-3007 was conducted in HIV negative children (all but two malnourished) at a single center in Zambia, and study RM02-3008 was conducted in HIV positive, severely malnourished, hospitalized children at a single center in Zambia. All patients enrolled had diarrhea at the time of enrollment (criteria given in Table 2) and oocysts of *C. parvum* had been identified in a stool sample collected within 7 days prior to enrollment. Parasitological diagnosis was reconfirmed at baseline in studies RM02-3007 and RM02-3008 (but not in Study RM-NTZ-98-002). Patients in these two studies for whom the diagnosis was not reconfirmed were excluded from the efficacy analyses.

Upon enrollment, the patients were randomized to either NTZ or Placebo arms, and received 3 consecutive days of treatment. In study RM-NTZ-98-002, randomization was stratified by age group (1-11 years, >11 years). A post-treatment physical exam was performed on day 7 (± 2 days). Two post-treatment fecal samples were obtained from each patient between day 7 and 10 (± 2 days). Each fecal sample was subjected to a parasitological exam. The criteria for evaluating clinical (Well, Continuing Illness, Clinical Failure) and parasitological (Eradication, Persistence) responses were clearly stated in the protocol. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. However, patients with other intestinal parasitic infections were not excluded from the study, due to high prevalence of intestinal parasitic infections in the Nile Delta Region and the potential difficulties in enrolling patients infected by *C. Parvum* alone.

The sample size for each of these studies was calculated (with an estimated 20% drop out rate) to provide 80% power to detect a difference between an 85% response rate for the active treatment group and a 40% response rate for the placebo group using a one sided alpha of 0.025. Study RM-NTZ-98-002 was powered to detect a difference in adults as well as children.

3.1.2 EFFICACY ENDPOINTS

All three studies evaluated two efficacy endpoints: **clinical response** (well, continuing illness, clinical failure) on study day 7 (4 days after the end of treatment) and **parasitological response** (eradication or persistence) based on two stool samples collected between study days 7 and 10. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours'

or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

In Study RM-NTZ-98-002, both of these endpoints were stated as primary endpoints, where as in Studies RM02-3007 and RM02-3008, clinical response was stated as primary endpoint and parasitological response was stated as secondary endpoint. Time from initiation of treatment to passage of last unformed stool (as reported by the patient in a diary) was evaluated as secondary efficacy endpoint in all three studies.

Reviewer's Comments

*1> Since the sponsor is seeking indications of "Treatment of Diarrhea". [redacted] **both clinical response and parasitological response are treated as primary endpoints in this review.***

2> It was stated in the protocol for study RM-NTZ-98-002 that [redacted] would be analyzed as a secondary endpoint. However, this was not done in the final study report. No explanation for this change was given. The data sets submitted with the application did not contain any information about this endpoint. Hence this review does not contain any discussion of the results concerning this endpoint. This endpoint was not considered in any of the other protocols in this NDA

3.1.3 STATISTICAL METHODOLOGY USED BY THE SPONSOR

Fisher's exact tests were used to compare the treatment groups in terms of clinical and parasitological response rates. The median time from initiation of therapy to date of last unformed stool was compared for the active and placebo treatment groups using Kaplan-Meier survival analysis. The sponsor also used the Median test for the observed data to test the null hypothesis that the two treatment groups have the same median time to last unformed stool.

Reviewer's Comment

Median test is a chi-squared test applied to the 2 x 2 contingency table constructed in such a way that the two entries in the first column are the number of observations for treatment 1 (say placebo) that are above and below the grand median (the median of all observations the both treatment groups combined), and the two entries in the second column are the number of observations for treatment 2 (say NTZ) that are above and below the grand median. For more discussion of this test the reader is referred to the reference (4).

3.1.4 DIFFERENCES/SIMILARITIES IN THE THREE STUDIES

The following table shows differences/similarities among the three studies in terms of location, study design, patient population and other characteristics.

Table 2: Controlled Clinical Trials for *C. Parvum*

	RM-NTZ-98-002	RM02-3007	RM02-3008
Center	Egypt	Zambia	Zambia
Patient population	Adults (age ≥ 12 years) and Children (age < 12 years)	Children (age < 3 years), all but two malnourished	Children (age < 12 years), all malnourished
HIV status	Negative	Negative	Positive
Diarrhea	> 4 stools /day	≥3 stools per day	≥3 stools per day
Parasitological diagnosis	Not confirmed at Baseline (enrollment)	Confirmed at Baseline(enrollment)	Confirmed at Baseline(enrollment)
Excluded from efficacy analyses	Patients with Bacterial infections	1> Patients with bacterial infections 2> Patients with no oocysts at baseline	1> Patients with bacterial infections 2> Patients with no oocysts at baseline

3.1.5 PATIENT DISPOSITION – ALL THREE STUDIES

Table 3: Patient disposition for Controlled clinical trials for *C. Parvum*

	RM-NTZ-98-002		RM02-3007		RM02-3008	
SCREENED	725		Not Reported		Not Reported	
	NTZ	Placebo	NTZ	Placebo	NTZ	Placebo
ENROLLED	50	50	25	25	25	25
COMPLETED	49	50	25	19	20	21
WITHDRAWN						
Withdrew Consent	1	0	None		None	
No baseline parasite	Not Tested		0	3*	None	
AE	None		0	1	None	
Death	None		0	4	5	4
OTHER INFECTIONS						
Bacterial	0	1	None		0	1
Parasitic	13	7	1	0	None	
PROTOCOL DEVIATIONS						
C. Parvum diagnosis more than 7 days prior to enrollment	9	11	None		None	
Late or No show for Post Rx exam Day 7	4	4	None		None	
Late or No show for Post Rx exam Day 10	6	12	1	1	None	

* one patient was withdrawn by investigator and 2 remained in the study

3.1.6 DEMOGRAPHICS AND BASELINE CHARACTERISITCS – ALL THREE STUDIES

The sponsor **summarized** demographic and disease related characteristics for all the subjects in each study regardless of their treatment assignment and noted that statistically significantly higher number of children reported liquid stools compared to adults.

Reviewer's Comment:
This reviewer summarized demographic and disease related characteristics in the following tables by the treatment group. These were generally comparable across the treatment groups.

Table 4A: Demographics and Baseline Characteristics - Adults

ADULTS						
Demographic or Disease Related Characteristics	**STUDY**					
	RM-NTZ-98-002		**RM02-3007**		**RM02-3008**	
	NTZ	**Placebo**	**NTZ**	**Placebo**	**NTZ**	**Placebo**
Male	12	15	ADULTS WERE NOT STUDIED IN THESE TRIALS			
Female	13	10				
Age (yrs)– Mean (s.d.)	35.52 (12.44)	34.92 (12.62)				
Weight (kgs)– Mean (s.d.)	70.60 (15.24)	73.56 (14.37)				
Stool Frequency						
5-10 stools /day	25	25				
> 10 stools /day	0	0				
Stool Consistency						
Liquid	9	2				
Semisolid	16	23				
Duration of Diarrhea- Median no. of days	9	10				

Table 4B: Demographics and Baseline Characteristics - Children

CHILDREN						
Demographic or Disease Related Characteristics	STUDY					
	RM-NTZ-98-002		RM02-3007		RM02-3008	
	NTZ	Placebo	NTZ	Placebo	NTZ	Placebo
Male	13	16	16	18	12	15
Female	12	9	9	7	13	10
Age (yrs)– Mean (s.d.)	5.34 (3.27)	5.0 (3.20)	1.24 (.44)	1.2 (.41)	21.76 (7.03)	27.04 (15.68)
Weight (kgs)– Mean (s.d.)	20.25 (7.95)	18.36 (8.17)	8.37 (1.53)	8.28 (1.28)	7.13 (1.49)	7.96 (2.7)
Stool Frequency						
3-4 stools/day	0	0	2	3	0	0
5-10 stools /day	23	23	23	21	24	25
> 10 stools /day	2	2	0	1	1	0
Stool Consistency						
Liquid	10	18	17	20	15	20
Semisolid	15	7	4	2	4	2
Liquid & Semisolid	0	0	4	3	5	3
Duration of Diarrhea- Median no. of days	9	9	18	10.5	29	21
CD4 count - Mean (s.d.)	N/A	N/A	N/A	N/A	618.59 (445.55)	620.81 (631.66)

3.1.7 SPONSOR'S EFFICACY RESULTS

3.1.7.1 Study RM-NTZ-98-002

Out of 100 patients enrolled in the study, the parent of one child randomized to NTZ decided not to participate in the study and returned all of the study medication on the day of enrollment. This patient was excluded from all efficacy analyses conducted by the sponsor. Also the sponsor excluded one patient (patient number 33C) randomized to placebo with a positive coproculture for bacterial cause of diarrhea from the analysis of clinical response, in accordance with the protocol. The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The sponsor stated that precise median time to last unformed stool for the placebo group could not be determined since patients were not followed up past the date of post-treatment examination (day 6).

Table 5. Sponsor's Efficacy results for Study RM-NTZ-98-002 – ITT population

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Median time to last unformed stool
ADULTS			
NTZ Placebo p-value (one sided)	18/25 (72%) 11/25 (44%) .0423	15/25 (60%) 6/25 (24%) .0104	3 days 6 days .0493
CHILDREN			
NTZ Placebo p-value (one sided)	21/24 (88%) 9/24 (38%) .0004	18/24 (75%) 5/25 (20%) .0001	3.5 days 6 days .0001

Reviewer's Comments:

1> The application consists of two NDA' — for NTZ tablets for adults and 21-498 for NTZ pediatric suspension for children. This reviewer will treat "Adults" and "Children" as two different patient populations and will review the data separately. This is consistent with the study design as the study was powered for adults and children separately.

2> The p-values in the above Table 5 are one-sided and statistical significance is concluded if p-value < 0.025.

3> For Adults (ITT population), the data did not provide statistically significant evidence of a difference between placebo and NTZ in terms of clinical response and median time to last unformed stool (p-values are > 0.025).

4> The sponsor also conducted "per protocol" analyses and results were consistent with those in Table 5.

5> This reviewer conducted several analyses to evaluate the evidence based on age group in children, gender, and patients with only C. Parvum infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.1.8 and 3.3).

3.1.7.2 Study RM02-3007

Out of 50 patients enrolled in the study, 3 patients (all in placebo group, all male) with no oocysts of *C. Parvum* in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population (consisting of 47 patients). The time to last unformed stool was compared using Kaplan-Meier curves and log-rank test.

Table 6. Sponsor's Efficacy results for Study RM02-3007 – ITT population

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Time to last unformed stool
CHILDREN			
NTZ	14/25 (56%)	13/25 (52%)	Can't be determined - More than 50% censoring
Placebo	5/22 (23%)	3/22 (14%)	Can't be determined - More than 50% censoring
p-value (two sided)	.0362	.0069	.278

Reviewer's Comments:

1> In this study, there was statistically significant difference in the mortality rate within 7 days following enrollment between NTZ and Placebo (0% versus 18%, respectively).

2> The p-values in the above Table 6 are two sided and statistical significance is concluded if p-value< 0.05.

3> The sponsor's analysis of time to last unformed stool has an error. This reviewer re-analyzed the data and the correct p-value is 0.46. This correction does not change the conclusion of no statistically significant difference between NTZ and placebo groups in terms of Time to Last Unformed Stool.

4> This reviewer conducted several analyses to evaluate the evidence based on gender, and patients with only C. Parvum infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.1.8 and 3.3).

5> Out of 50 patients enrolled in this study, 3 patients (all in placebo group, all male) with no oocysts of C. Parvum in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. Since these patients were treated and the clinical response and parasitological response was observed for all three patients, this reviewer conducted sensitivity analyses by including these patients. In these analyses, statistical significance was not reached for both endpoints (see section 3.3.2 for details and further discussion).

3.1.7.3 Study RM02-3008

Out of 50 patients enrolled in the study, 1 patient (placebo group) with bacterial cause of diarrhea (Salmonella) was excluded by the sponsor from efficacy analyses of clinical response. The following table shows sponsor's primary analyses for the clinical and

parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The time to last unformed stool was compared using Kaplan-Meier curves and log-rank test.

Table 7. Sponsor's Efficacy results for Study RM02-3008 – ITT population

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Time to last unformed stool
CHILDREN			
NTZ	2/25 (8%)	4/25 (16%)	Can't be determined - More than 50% censoring
Placebo	6/24 (25%)	5/25 (20%)	Can't be determined - More than 50% censoring
p-value (two sided)	.1383	1.0	.07

Reviewer's Comment:

*1> **Statistically significant benefit of NTZ was not demonstrated in this study. No relationship between demographic or disease related characteristics and the efficacy endpoints was observed.***

*2> **The sponsor pointed out that aside from their infection with HIV, the patients enrolled in this study were significantly more malnourished (mean weight-for-age z-scores: -5.5 compared to -3.5, p<0.0001); they reported a longer duration of diarrhea at time of enrollment (49 days compared to 20 days, p< 0.01); and they had lower CD4 cell counts (mean: 620 cells/mm^3 compared to 1532 cells/mm^3, p<.0001) than HIV negative children enrolled in study RM02-3007.***

3.1.8 CORRELATION BETWEEN CLINICAL RESPONSE AND PARASITOLOGICAL RESPONSE

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1; 1] with higher positive values indicating higher concordance. The use of kappa to evaluate the correlation between clinical and parasitological response is discussed in Section 3.4.

In this application, for the NTZ arm, positive kappa values are expected in order to consider parasitological response to be a meaningful endpoint. For the placebo arm, near zero kappa values are expected since spontaneous resolution of symptoms is observed in patients with *C. Parvum* and the possibilities of diarrhea from infections other than *C. Parvum* infection exist.

Kappa Coefficients for all the three studies are provided in the following table.

Table 8: Kappa Coefficients

Study	Population	Treatment	N	Kappa	No. of discordant pairs (well & persistence + ill & eradicated)
RM-NTZ 98-002	Adults	Placebo	25	0.061	11 (8+3)
		NTZ	25	-0.312	15 (9+6)
RM-NTZ 98-002	Children (1-11 yrs)	Placebo	25*	0.039	10 (7+3)
		NTZ	24	0.333	5 (4+1)
RM02-3007	Children (< 4 yrs)	Placebo	22	0.096	6 (4+2)
		NTZ	25	0.116	11 (6+5)
RM02-3008	Children (1-11 yrs)	Placebo	25	0.311	5 (3+2)
		NTZ	25	0.250	4 (1+3)

* One patient excluded by the sponsor from analyses of clinical response in included in the calculation of kappa.

The negative Kappa for the NTZ arm in adults population in Study RM-NTZ-98-002 raises questions about meaningfulness of parasitological response as an efficacy endpoint. This together with statistically insignificant clinical response of NTZ compared to placebo for this population leads one to conclude that evidence of efficacy of NTZ for adults is not adequate.

Further analyses for the subgroups based on age group in children, gender and patients with only *C. Parvum* infection is contained in Section 3.3.

3.2 DIARRHEA CAUSED BY *GIARDIA LAMBLIA*

3.2.1 STUDY DESIGN

The sponsor conducted two randomized studies to evaluate efficacy of NTZ in the treatment of diarrhea caused by *G. Lamblia.* Dosing and study design information is given in Table 1.

Study ——— was a double-blind placebo-controlled study in adults (age 12-65 years). To be eligible for inclusion in the study, patients must have reported diarrhea (> 4 unformed bowel movements/day) and cysts or trophozoites of *Entamoeba histolytica/ dispar or Giardia Lamblia* must have been identified in a stool sample collected within 7 days prior to enrollment. The sample size for study ——— was calculated (with an estimated 40/ 60 distribution of patients with amoebiasis or giardiasis and 7% drop out rate) to provide 90% power to detect a difference between an 80% response rate for the active treatment group and a 25% response rate for the placebo group using a two sided alpha of 0.05. Upon enrollment, the patients were randomized to either NTZ or Placebo arms, and received 3 consecutive days of treatment. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. However, patients with other intestinal parasitic infections were not excluded from the study, due to high prevalence of intestinal parasitic infections in the Nile Delta Region and the potential difficulties in enrolling patients infected by *G.Lamblia* alone. This study was terminated early so that the principle investigator could take a planned leave of absence.

Study RM-NTZ-99-010 was single-blind (parasitologist blinded) active controlled study in children (age 2-11 years). Metrodidazole was used as control treatment. To be eligible for inclusion in the study, patients must have reported acute diarrhea (> 2 unformed bowel movements/day) or chronic diarrhea (unformed stools with or without increased frequency for more than 4 weeks) and positive immuno-assay for *Giardia Lamblia* in a stool sample collected within 7 days prior to enrollment. The sample size for study RM-NTZ-99-010 was calculated (with an estimated 8% drop out rate) to provide 80% power to detect a less than 20% difference between response rates for the active treatment group and the active control group using a two sided alpha of 0.05. It was assumed that response rates for both treatment groups were equal to 85% for this sample size calculation. Patients with stool sample positive for *Entamoeba histolytica* or *C. Parvum* were not enrolled in the study. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. Parasitological diagnosis was reconfirmed at baseline in this study and patients for whom the diagnosis was not confirmed were to be excluded from efficacy analyses.

A post-treatment physical exam was performed on day 7 (± 2 days). Two post-treatment fecal samples were obtained from each patient between day 7 and 10 (± 2 days). Each fecal sample was subjected to a parasitological exam. The criteria for evaluating clinical (Well, Continuing Illness, Clinical Failure) and parasitological (Eradication, Persistence) responses were clearly stated in the protocol.

3.2.2 EFFICACY ENDPOINTS

All three studies evaluated two efficacy endpoints: **clinical response** (well, continuing illness, clinical failure) on study day 7 (4 days after the end of treatment) and **parasitological response** (eradication or persistence) based on two stool samples collected between study days 7 and 10. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

In Study [redacted] both of these endpoints were stated as primary endpoints, where as in Study RM-NTZ-99-010 clinical response was stated as primary endpoint and parasitological response was stated as secondary endpoint.

Time from initiation of treatment to passage of last unformed stool (as reported by the patient in a diary) was evaluated as secondary efficacy endpoint in both studies.

In Study RM-NTZ-99-010, "Therapeutic Response" defined as simultaneous "Well" clinical response and "eradicated" parasitological response was also evaluated as a secondary endpoint.

Reviewer's Comment

*Since the sponsor is seeking indications of "Treatment of Diarrhea" [redacted] **both clinical response and parasitological response are treated as primary endpoints in this review.***

3.2.3 STATISTICAL METHODOLOGY USED BY THE SPONSOR

Fisher's exact tests were used to compare the treatment groups in terms of clinical and parasitological response rates. The median time from initiation of therapy to date of last unformed stool was compared for the two treatment groups using a median test.

Reviewer's Comment

Median Test is a chi-squared test applied to the 2 x 2 contingency table constructed in such a way that the two entries in the first column are the number of observations for treatment 1 (say placebo) that are above and below the grand median (the median of all observations the both treatment groups combined), and the two entries in the second column are the number of observations for treatment 2 (say NTZ) that are above and below the grand median. For more discussion of this test the reader is referred to the reference (4).

3.2.4 DIFFERENCES/SIMILARITIES IN THE TWO STUDIES

The following table shows differences/similarities among the two studies in terms of location, study design, patient population and other characteristics.

Table 9: Controlled Clinical Trials for *G. Lamblia*

		RM-NTZ-98-010
Center	Egypt	Peru
Patient population	Adults (age ≥ 12 years) with Ameobiasis or Giadiasis	Children (age 2-11 years) with Giardiasis only.
Control Treatment	Placebo	Metrodinazole
Blind	Double-blind	Parasitologist blinded
Diarrhea	> 4 unformed stools /day	≥3 unformed stools per day OR unformed stools with or without increased frequency for more than 4 weeks.
Parasitological diagnosis	Not confirmed at Baseline (enrollment)	Confirmed at Baseline (enrollment)
Excluded from efficacy analyses	Patients with Bacterial infections	1> Patients with bacterial infections 2> Patients with no cysts at baseline

3.2.5 PATIENT DISPOSITION

The following table gives an overview of the patient disposition including drop-outs and protocol deviations for both studies.

Table 10: Patient disposition for Controlled clinical trials for *G. Lamblia*

			RM-NTZ-99-010	
	NTZ	**Placebo**	**NTZ**	**Metronidazole**
ENROLLED	48	45	55	55
COMPLETED	47	41	55	53
WITHDRAWN				
Withdrew Consent	1	1	0	0
Loss to follow-up	0	1*	0	2*
Error in Diagnosis	0	1	0	0
Error in Diagnosis and Loss to Follow-up	0	1	0	0
No baseline parasite	Not Tested		0	0
Death	0	1	0	0
OTHER INFECTIONS				
Parasitic	9	8	11	8
PROTOCOL DEVIATIONS				
Concomitant Medication	0	0	1	0
Late or No show for Post Rx exam Day 7	5	4	1	0

*Patients treated as failures for efficacy analyses

3.2.6 DEMOGRAPHICS AND BASELINE CHARACTERISITCS

The sponsor summarized demographic and disease related characteristics for all the subjects in each study by their treatment assignment. These were generally comparable across the treatment groups.

Table 11: Demographics and Baseline Characteristics

Demographic or Disease Related Characteristic	STUDY			
	———		RM-NTZ 99-010	
	NTZ	Placebo	NTZ	Metronidazole
Male	31	26	27	27
Female	16	16	28	28
Age (yrs)– Mean (s.d.)	34.5 (17.7)	29.9 (14.4)	5.51 (2.53)	5.84 (2.61)
Weight (kgs)– Mean (s.d.)	63.9 (16.6)	58.4 (18.6)	21.45 (7.86)	20.87 (5.76)
Stool Frequency				
3-10 stools /day	41	36	53	55
> 10 stools /day	5	6	2	0
Stool Consistency				
Liquid	16	16	23	15
Semisolid	31	26	30	38
Liquid & Semisolid	0	0	2	2
Duration of Diarrhea- Median no. of days	9	9	13	18
Abdominal abnormalities	36	32	17	19

For study ——— , the sponsor provided demographic results for each of the two subgroups, Ameobiasis patients, and Giardiasis patients. Again these were generally comparable across the two treatment groups.

3.2.7 SPONSOR'S EFFICACY RESULTS

3.2.7.1 Study ———

Out of 93 patients enrolled in the study, two patients decided not to participate in the study and returned all of the study medication. These patients were excluded from all efficacy analyses conducted by the sponsor. Also the sponsor excluded two patients (patient numbers 81 and 83) who were enrolled without diagnosis of amoebiasis or giardiasis from all efficacy analysis. The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The sponsor stated that precise median time to last unformed stool for the placebo group

could not be determined since patients were not followed up past the date of post-treatment examination (day 6).

Table 12. Sponsor's Efficacy results for Study ——— ITT population

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Median time to last unformed stool
AMOEBIASIS PATIENTS			
NTZ	28/36 (78%)	25/36 (69%)	3 days
Placebo	13/31 (42%)	12/31 (39%)	6 days
p-value (two sided)	.0052	.0148	.0012 (Median test)
GIARDIASIS PATIENTS			
NTZ	14/17 (82%)	12/17 (71%)	3 days
Placebo	6/19 (32%)	0/19 (0%)	6 days
p-value (two sided)	.0031	<.0001	.0068 (Median test)

Reviewer's Comments:

1> In this study, 17 Giardiasis patients (9-NTZ, 8-placebo) had other additional infections besides Giardiasis. When patients infected with only Giardia Lamblia infection were isolated, the sample size was reduced to 18 patients, of whom, only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes are very small and hence the results of this study, although statistically significant, can not be generalized.

2> This reviewer also investigated the correlation between clinical and parasitological response (See Sections 3.2.8 and 3.3) and the subgroup of patients with only Giardiasis (and not Amoebiasis) (See Section 3.3.4).

3.2.7.2 Study RM-NTZ-99-010

The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population (consisting of all 110 patients enrolled in the study). The 95% confidence interval for the difference is calculated with correction for continuity. The time to last unformed stool was compared using Median Test. In this study the sponsor also analyzed "therapeutic" response defined as simultaneous occurrence of both "well" clinical response and "eradicated" parasitological response.

Table 13. Sponsor's Efficacy results for Study RM-NTZ-99-010 – ITT population

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Therapeutic Response	Median Time to last unformed stool (No. of patients with data)
CHILDREN				
NTZ	47/55 (85%)	39/55 (71%)	36/55 (65%)	4 days (23/55)
Metrondazole	44/55 (80%)	41/55 (75%)	35/55 (64%)	4 days (12/55)
p-value (two sided)	.6148	.8307	1.000	.3799 (Median test)
Difference 95% CI	5% (-10% , 21%)	4% (-21%, 14%)	1% (-17%, 20%)	

Reviewer's Comments:

1> ***The results in the above table support the sponsor's claim that clinical response rate for NTZ is no more than 20% inferior to that of metronidazole. However, since the confidence interval for the difference in parasitological response rates includes a lower bound of –20%, it does not provide sufficient evidence of non-inferiority of NTZ compared to metronidazole in terms of parasitological response.***

2> ***The sponsor also conducted "per protocol" analyses for the subset of patients who took all their study medication and returned for follow-up. For these analyses 17 patients were excluded from the ITT population for protocol violations(8 –NTZ, 9-Metronidazole).These results were consistent with those for the ITT population in the above table.***

3> ***This reviewer conducted several analyses to evaluate the evidence based on gender, and patients with only G. Lamblia infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.2.8 and 3.3).***

3.2.8 CORRELATION BETWEEN CLINICAL RESPONSE AND PARASITOLOGICAL RESPONSE

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1, 1]. The use of kappa to evaluate the correlation between clinical and parasitological response is discussed in Section 3.4.

In this application, for the NTZ arm, positive kappa values are expected in order to consider parasitological response to be a meaningful endpoint. Kappa Coefficients for all the two Giardia studies are provided in the following table.

Table 14: Kappa Coefficients

Study	Population	Treatment	N	Kappa	No. of discordant pairs (well & persistence + ill & eradicated)
RM-NTZ- [redacted]	Adults (Giardiasis)	NTZ	17	-.283	8 (5+3)
		Placebo	19	N/A	6 (6+0)
RM-NTZ-99-010	Children (2-11 yrs)	NTZ	55	.276	14 (11+3)
		Metronidazole	55	.227	15 (9+6)

The negative Kappa for the NTZ arm in adults population in Study [redacted] raises questions about meaningfulness of parasitological response as an efficacy endpoint. This together with small sample size of the group of patients with Giardiasis only (11-NTZ, 11-placebo) leads one to conclude that evidence of efficacy of NTZ for adults with Giardiasis is not adequate.

Further analyses for the subgroups based on age group in children, gender and patients with only *G. Lamblia* infection is contained in Section 3.3.

3.3 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

In this section we evaluate findings in subgroup/special populations for studies RM-NTZ-98-002 and RM02-3007 in patients with *C. Parvum* infection and for studies — and RM-NTZ-99-010 in patients with *Giardia Lamblia* infection. These analyses are post-hoc and the sample sizes of some of the subgroups are small resulting in substantial decrease in power to detect treatment differences.

Study RM02-3008 in patients with *C. Parvum* infection failed to show any evidence of drug benefit. Therefore subgroup analyses were not carried out for this study. Study RM-NTZ-— had a very small sample of patients with *Giardia Lamblia* infection. Therefore subgroup analyses based on gender was not carried out for this study.

3.3.1 STUDY RM-NTZ-98-002

The following tables show the reviewer's results for the subgroups of the ITT population based on gender and age group of children.

Table 15: **Adult ITT population Subgroups in Study RM-NTZ-98-002**

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
ADULTS				
NTZ Placebo p-value (two sided)	18/25 (72%) 11/25 (44%) .0845	15/25 (60%) 6/25 (24%) .0209	-0.316 (.162) 0.061 (.182)	15 (9+6) 11 (8+3)
ADULTS – FEMALE				
NTZ Placebo p-value (two sided)	11/13 (84.6%) 4/10 (40%) .0393	7/13 (53.9%) 2/10 (20 %) .1968	-0.3 (.181) 0.091 (.288)	8 (6+2) 4 (3+1)
ADULTS – MALE				
NTZ Placebo p-value (two sided)	7/12 (58.3%) 7/15 (46.7%) .7036	8/12 (66.7%) 4/15 (26.7%) .0574	-0.235 (.264) 0.037 (.236)	7 (3+4) 7 (5+2)

These analyses show a consistent trend of numerically higher percents for the NTZ arm compared to the placebo arm. However, the differences generally are not statistically significant. Moreover, kappa coefficient is consistently negative for the NTZ arm raising doubts about the meaningfulness of one of the two endpoints. Efficacy of NTZ has not been adequately demonstrated for the population of adults.

Table 16: **Children** ITT *population Subgroups in Study RM-NTZ-98-002

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
CHILDREN				
NTZ Placebo p-value (two sided)	21/24 (87.5%) 9/25 (36%) .0003	18/24 (75%) 5/25 (20%) .0002	.333 (.262) .039 (.188)	5 (4+1) 10 (7+3)
CHILTDREN – FEMALE				
NTZ Placebo p-value (two sided)	12/12 (100%) 5/9 (55.6%) .0211	9/12 (75%) 2/9 (22.2%) .03	N/A -.047 (.262)	3 (3+0) 5 (4+1)
CHILDREN – MALE				
NTZ Placebo p-value (two sided)	9/12 (75%) 4/16 (25%) .02	9/12 (75%) 3/16 (18.8%) .0061	.556 (.278) .091 (.262)	2 (1+1) 5 (3+2)
CHILDREN – AGE < 4				
NTZ Placebo p-value (two sided)	10/11 (90.9%) 4/11 (36.4%) .024	8/11 (72.7%) 3/11 (27.3%) .086	-.158 (.128) .377 (.29)	4 (3+1) 3 (2+1)
CHILDREN – AGE 4-11				
NTZ Placebo p-value (two sided)	11/13 (84.6%) 5/14 (35.7%) .018	10/13 (76.9%) 2/14 (14.3%) .002	.755 (.228) -.256 (.144)	1 (1+0) 7 (5+2)

*** Includes one child in the placebo arm who was excluded from analysis of clinical response by the sponsor due to bacterial cause of diarrhea.**

For the population of children, statistically significant higher percent of response is observed for the NTZ arm compared to the placebo arm. Also kappa coefficients are consistently positive (with the exception of the subgroup of children < 4 years of age). Efficacy of NTZ is adequately demonstrated for the population of children in this study.

In this study, 21 patients had mixed infections of whom 13 were children (4 placebo, 9 NTZ) and 8 were adults (4 placebo, 4 NTZ). Since these patients may potentially introduce bias, this reviewer conducted sensitivity analyses excluding these patients from the ITT population. The following tables show that the efficacy results for this population of patients

with only *C. Parvum* infection and for the subgroups of this population are consistent with the results for the ITT population and its subgroups.

Table 17: Adult *C. Parvum-only* population subgroups in Study RM-NTZ-98-002

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
ADULTS				
NTZ	15/21 (71.4%)	12/21 (57.1%)	-0.319 (.181)	13 (8+5)
Placebo	9/21 (42.9%)	6/21 (28.6%)	0.087 (.209)	9 (6+3)
p-value (two sided)	.118	.118		
ADULTS – FEMALE				
NTZ	11/13 (84.6%)	7/13 (53.9%)	-0.3 (.181)	8 (6+2)
Placebo	3/8 (37.5%)	2/8 (25 %)	0.143 (.347)	3 (2+1)
p-value (two sided)	.056	.367		
ADULTS – MALE				
NTZ	4/8 (50%)	5/8 (62.5%)	-0.25 (.332)	5 (2+3)
Placebo	6/13 (46.2%)	4/13 (30.8%)	0.049 (.264)	6 (4+2)
p-value (two sided)	1.0	.203		

Table 18: Children *C. Parvum-only* population subgroups in Study RM-NTZ-98-002

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
CHILDREN				
NTZ	13/15 (86.7%)	11/15 (73.3%)	0.189 (.270)	4 (3+1)
Placebo	6/21 (28.6%)	4/21 (19.1%)	-0.037(.204)	8 (5+3)
p-value (two sided)	.0008	.0019		
CHILTDREN – FEMALE				
NTZ	8/8 (100%)	6/8 (75%)	N/A	2 (2+0)
Placebo	4/8 (50%)	2/8 (25%)	0.0 (.306)	4 (3+1)
p-value (two sided)	.0769	.1319		
CHILDREN – MALE				
NTZ	5/7 (71.4%)	5/7 (71.4%)	0.3 (.391)	2 (1+1)
Placebo	2/13(15.4%)	2/13(15.4%)	-0.182 (.089)	4 (2+2)
p-value (two sided)	.0223	.0223		
CHILDREN – AGE < 4				
NTZ	6/7 (85.7%)	4/7 (57.1%)	-0.273 (.230)	4 (3+1)
Placebo	2/9 (22.2%)	2/9 (22.2%)	0.357 (.367)	2 (1+1)
p-value (two sided)	.041	.302		
CHILDREN – AGE 4-11				
NTZ	7/8 (87.5%)	7/8 (87.5%)	1.0 (N/A)	0 (0+0)

Placebo	4/12 (33.3%)	2/12 (16.7%)	-0.286 (.150)	6 (4+2)
p-value (two sided)	.028	.005		

3.3.2 STUDY RM02-3007

Out of 50 patients enrolled in this study, 3 patients (all in placebo group, all male) with no oocysts of *C. Parvum* in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. Since these patients were treated and the clinical response and parasitological response was observed for all three patients, this reviewer conducted sensitivity analyses by including these patients. One may recall that in the other similarly designed study (RM-NTZ-98-002) in HIV negative children with *C. Parvum* infection, patients were not tested for oocysts of *C. Parvum* at baseline, and therefore no one was excluded from that study due to absence of oocysts of *C. Parvum* at baseline.

The following are the results of the reviewer's sensitivity analyses.

Table 19. Efficacy results for Study RM02-3007 – All Patients

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
CHILDREN				
NTZ	14/25 (56%)	13/25 (52%)	0.116 (.198)	11 (6+5)
Placebo	8/25 (32%)	6/25 (24%)	0.409 (.198)	6 (4+2)
p-value (two sided)	.1536	.0792		
CHILDREN- FEMALE				
NTZ	7/9 (77.8%)	6/9 (66.7%)	0.182 (.336)	3 (2+1)
Placebo	1/7 (14.3%)	0/7 (0%)	N/A	1 (1+0)
p-value (two sided)	.0406	.0114		
CHILDREN – MALE				
NTZ	7/16 (43.8%)	7/16 (43.8)	-0.016 (.25)	8 (4+4)
Placebo	7/18 (38.9%)	6/18 (33.3%)	0.4 (.222)	5 (3+2)
p-value (two sided)	1.0	.7254		

In these analyses, statistical significance is not reached for both endpoints for the population of all children as well as for the subgroup of male children.

In the sponsor's ITT analyses in Table 6, statistical significance is reached for both endpoints for the ITT population of all children. However, when these analyses are carried out for the gender based subgroups of ITT population, the statistical significance is reached

for the subgroup of female children, but not for the subgroup of male children (consistent with Table 17).

3.3.3 STUDIES RM-NTZ-98-002 AND RM02-3007 POOLED

This reviewer analyzed the pooled data on all children to evaluate the collective evidence for children with *C. Parvum* infection. The pooling of data was done under certain restrictions suggested by the medical officer reviewing this application. These restrictions are as follows:

1> Children who had other infections in addition to *C. Parvum* infection and/or did not take the medication were excluded

2> All children whose stool samples tested positive for oocysts of only *C. Parvum* at the screening visit were included.

The results of these analyses are as follows:

Table 20: Children *C. Parvum-only* population – Pooled data

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
ALL DATA				
Placebo	14/46 (30.4%)	10/46 (21.7%)	0.218 (.153)	14 (9+5)
NTZ	26/39 (66.7%)	23/39 (59.0%)	0.182 (.158)	15 (9+6)
p-value (two sided)	.0011	.0007		
AGE < 4 YEARS				
Placebo	10/34 (29.4%)	8/34 (23.5%)	0.398 (.175)	8 (5+3)
NTZ	19/31 (61.3%)	16/31 (51.6%)	0.025 (.176)	15 (9+6)
p-value (two sided)	.0131	.0231		
AGE 4-11 YEARS (only in Study RM-NTZ-98-002)				
Placebo	4/12 (33.3%)	2/12 (16.7%)	-0.286 (.15)	6 (4+2)
NTZ	7/8 (87.5%)	7/8 (87.5%)	1.0 (N/A)	0 (0+0)
p-value (two sided)	.0281	.0045		
FEMALE				
Placebo	5/15 (33.3%)	2/15 (13.3%)	0.118 (.236)	5 (4+1)
NTZ	14/16 (87.5%)	11/16 (68.8%)	0.130 (.235)	5 (4+1)
p-value (two sided)	.0032	.0032		
MALE				
Placebo	9/31 (29.0%)	8/31 (25.8%)	0.271 (.188)	9 (5+4)
NTZ	12/23 (52.2%)	12/23 (52.2%)	0.129 (.207)	10 (5+5)
p-value (two sided)	.0994	.0861		
AFRICAN – STUDY RM02-3007				
Placebo	8/25 (32%)	6/25 (24%)	0.409 (.198)	6 (4+2)
NTZ	13/24 (54.2%)	12/24 (50%)	0.083 (.203)	11 (6+5)
p-value (two sided)	.1536	.0792		
CAUCASIAN – STUDY RM-NTZ-98-002				
Placebo	6/21 (28.6%)	4/21 (19.1%)	-0.037 (.204)	8 (5+3)

NTZ	13/15 (86.7%)	11/15 (73.3%)	0.189 (.270)	4 (3+1)
p-value (two sided)	.0008	.0019		

These results show a consistent clinical response rate for placebo ranging from 28.6% to 33.3%. However the clinical response rate for NTZ is highly variable ranging from 52.2% to 87.5%. Parasitological response rate varies greatly for both placebo as well as NTZ. Correlation between clinical and parasitological response rates as measures by kappa coefficient is inconsistent. For those subgroups, where statistical significance is not reached, the trend in both clinical and parasitological response is in favor of the test drug NTZ. In summary, efficacy of NTZ in children with *C. Parvum* infection is demonstrated in terms of clinical response endpoint, but there is lack of robustness in terms of parasitological response endpoint.

3.3.4 STUDY

In this study, patients (age ≥ 12 years) were enrolled with diagnosis of either Amoebiasis or Giardiasis or both. Since the sponsor is seeking indication of Giardiasis only, the reviewer obtained the following results for the subgroups based on the type of infection.

Table 21: Adult population subgroups in Study

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well & Persistence+ Ill & Eradicated)
ONLY GIARDIASIS				
Placebo	4/11 (36.4%)	0/11 (0%)	N/A	4 (4+0)
NTZ	10/11 (90.9%)	9/11 (81.8%)	-.138 (.100)	3 (2+1)
p-value (two sided)	.024	.0002		
GIARDIASIS + AMOEBIASIS				
Placebo	2/8 (25.0%)	0/8 (0%)	N/A	2 (2+0)
NTZ	4/6 (66.7%)	3/6 (50%)	-.667 (.287)	5 (3+2)
p-value (two sided)	.287	.055		
ALL GIARDIASIS				
Placebo	6/19 (31.6%)	0/19 (0%)	N/A	6 (6+0)
NTZ	14/17 (82.4%)	12/17 (70.6%)	-.283 (.115)	8 (5+3)
p-value (two sided)	.003	<.0001		

Although statistically significant clinical and parasitological response in demonstrated, the sample size is very small for the subgroup of patient with only Giardiasis and not Amoebiasis (11 treated with NTZ, only 3 female). Also 17 patients (9-NTZ, 8-placebo) had other additional infections besides Giardiasis. In this study, when patients infected with only *Giardia Lamblia* infection were isolated, the sample size was reduced to 18 patients, of whom,

only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes were very small and hence the results of this study, although statistically significant, could not be generalized. In addition kappa coefficients for all NTZ arms are negative implying poor correlation between clinical and parasitological endpoints. Thus, efficacy of NTZ for the population of adults with Giardiasis has not been adequately demonstrated.

3.3.5 STUDY RM-NTZ-99-010

In this study in children conducted at a single center in Peru, 110 patients were enrolled of whom 25 patients (11- Metronidazole, 14 -NTZ) were 2-3 years of age and 85 patients (44 - Metronidazole, 41-NTZ) were 4-11 years of age. All patients were diagnosed with Giardiasis re-confirmed at baseline. ITT population consisted of all 110 patients. The following table shows the results of the reviewer's analyses for the subgroups of ITT population based on the age group and gender. The continuity correction is not used in the calculation of 95% confidence intervals.

Table 22: Children ITT population Subgroups in Study RM-NTZ-99-010

Study Medication	"Well" Clinical Response	"Eradicated" Parasitological Response	Kappa (s.d.)	No of discordant pairs (Well& Persistence+ Ill & Eradicated)
ALL CHILDREN				
Metronidazole	44/55 (80%)	41/55 (74.6%)	.227 (.147)	15 (9+6)
NTZ	47/55 (85.5%)	39/55 (70.9%)	.276 (.139)	14 (11+3)
95% CI*	(-8.64, 19.54)	(-20.3, 13.0)		
CHILTDREN – FEMALE				
Metronidazole	23/28 (82.1%)	21/28 (75%)	.368 (.206)	6 (4+2)
NTZ	25/28 (89.3%)	19/28 (67.7%)	.404 (.173)	6 (6+0)
95% CI*	(-11.04, 25.44)	(-30.91, 16.31)		
CHILDREN – MALE				
Metronidazole	21/27 (77.8%)	20/27 (74.1%)	.09 (.201)	9 (5+4)
NTZ	22/27 (81.5%)	20/27 (74.1%)	.15 (.205)	8 (5+3)
95% CI*	(-17.75, 25.15)	(-23.37, 23.37)		
CHILDREN – AGE < 4				
Metronidazole	9/11 (81.8%)	9/11 (81.8%)	.389 (.353)	2 (1+1)
NTZ	13/14 (92.9%)	9/14 (64.3%)	.243 (.209)	4 (4+0)
95% CI*	(-15.44, 37.52)	(-51.43, 16.37)		
CHILDREN – AGE 4-11				
Metronidazole	35/44 (79.6%)	32/44 (72.7%)	.192 (.161)	13 (8+5)
NTZ	34/41 (82.9%)	30/41 (73.2%)	.298 (.168)	10 (7+3)
95% CI*	(-13.19, 19.95)	(-18.46, 19.34)		

*** 95% Confidence Interval for the difference (NTZ-Metronidazole) - continuity correction not used**

The above analyses show that for all the subgroups, the non-inferiority of NTZ compared to metronidazole with a margin of 20% is demonstrated with 95% confidence in terms of the clinical response rates, but not in terms of the parasitological response rates.

These results for the subgroups are consistent with those for the ITT population. This reviewer also carried out analyses for the "Per protocol" population (17 patients excluded: 8-NTZ, 9-Metronidazole) and for the population of patients with no infections other than infection with *Giardia Lamblia* (19 patients excluded for mixed infections: 11-NTZ, 8-Metronidazole). These results (not shown here) were also consistent with those for the ITT population.

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3.4 STATISTICAL AND TECHNICAL ISSUES

3.4.1 KAPPA COEFICIENT

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1, 1]. In statistical literature, a guideline for assessing agreement between two endpoints has been published (2) and is as follows: For $0 \leq$ kappa < 0.4, there is at best marginal agreement, for $0.4 \leq$ kappa < 0.75, the agreement is good and for kappa ≥ 0.75, there is excellent agreement between the two endpoints.

In this section, we discuss the use of Kappa to evaluate correlation between clinical response and parasitological response. The following table shows concordant and discordant pairs of responses.

Table 23: Concordance and Discordance

		Clinical Response	
		Well	*Continuing Illness*
Parasitological Response	*Eradicated*	Concordance	Discordance
	Persistence	Discordance	Concordance

Kappa close or equal to +1.0 implies that most patients experiencing "well" clinical response are also experiencing "Eradicated" parasitological response or vice a versa (that is most patients in "Continuing Illness" clinical response category are also in "persistence" parasitological response category). In this case, the number of discordant pairs is close to zero and both clinical response and parasitological response are equally effective in assessing drug benefit, and either one would suffice as a primary endpoint for evaluation of efficacy.

Table 24: Hypothetical Example of Kappa = 1.0

DRUG A		Clinical Response		
		Well	*Continuing Illness*	*Total*
Parasitological Response	*Eradicated*	10	0	10 (40%)
	Persistence	0	15	15
	Total	10 (40%)	15	25

Kappa close or equal to 0.0 implies that the two endpoints are independent. That is probability of "well" clinical response given "eradicated" parasitological response and probability of "well" clinical response given "persistence" parasitological response are same and equal the overall (unconditional) probability of "well" clinical response. In this case the

number of concordant pairs is approximately same as the number of discordant pairs, and simultaneous (joint) evaluation both endpoints may be needed to assess the drug benefit.

Table 25: Hypothetical Example of Kappa = 0.0

DRUG B		Clinical Response		
		Well	*Continuing Illness*	*Total*
Parasitological Response	*Eradicated*	4	6	10 (40%)
	Persistence	6	9	15
	Total	10 (40%)	15	25

Kappa close or equal to –1.0 implies that most patients experiencing "well" clinical response are in "persistence" parasitological response category and vice a versa. In this case the number of discordant pairs is very high and the drug benefit is not reproducible when assessed through these endpoints. One has to use caution in the evaluation of efficacy of the drug in this situation as one of the two endpoints may be meaningless in the assessment of efficacy.

Table 26: Hypothetical Example of Kappa* ≅ -1.0

DRUG C		Clinical Response		
		Well	*Continuing Illness*	*Total*
Parasitological Response	*Eradicated*	0	10	10 (40%)
	Persistence	15	0	15
	Total	15 (60%)	10	25

* **exact value of Kappa is - 0.92**

For the sake of simplicity, let us disregard the issue of statistical significance and look at the percent rates. From the three tables 24, 25 and 26 above, one can easily see that even though Drug C produced best (60%) clinical response and all three drugs produced same (40%) parasitological response, one can not conclude that Drug C is better than Drugs A and B because of varying patterns of concordance and discordance quantified by Kappa coefficients.

These tables illustrate how difficult it is to interpret the results when varying values of kappa are observed for different treatment arms in the same clinical trial.

For a further discussion, this reviewer recommends an excellent literature review of kappa coefficient published in 2002(3).

3.5 STATISTICAL EVALUATION OF COLLECTIVE EVIDENCE

An overall evaluation of efficacy based on the strength of statistical evidence from each of the five well controlled pivotal studies in this application and a collective assessment of drug performance for all five studies is addressed in the previous sections and is not repeated here. Fully informative tables and text to collectively assess the evidence have also been provided in the previous sections. The overall extent to which study results support the efficacy claim is outlined in Section 3.6 (and also in section 1.1 of Executive summary).

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3.6 CONCLUSIONS AND RECOMMENDATIONS

Single Center Studies

All five studies in this application were designed as single center, single race studies conducted outside of Unites States. This fact seriously compromises the generalizability of the results of these studies to the population in Unites States, and applicability of these results to future non-inferiority studies where NTZ, if approved, may be used as an active comparator. The data from these studies could not be used to assess the effects of important covariates such as race, age group, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

It is strongly recommended that any future studies in the development of NTZ be designed and conducted as adequate, well-controlled, **multi-center** studies.

C. Parvum

Adult Studies

Adult HIV negative population (age $\geq$ 12 years) was studied in only one study, RM-NTZ-98-002, conducted at a single center in Egypt. The difference between NTZ and placebo for the clinical endpoint was not statistically significant. Although statistical significance was demonstrated for the parasitological endpoint, poor correlation between clinical and parasitological endpoints for the NTZ arm (kappa = -0.312) seriously compromises the meaningfulness of parasitological endpoint for this population.

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum* [redacted] However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies

The population of HIV negative children (age 1-11 years) was studied in two randomized placebo controlled studies, one, RM-NTZ-98-002, conducted at a single center in Egypt and another, RM02-3007, conducted at a single center in Zambia.

In both studies, the differences between NTZ and placebo were statistically significant (in favor of NTZ) in terms of clinical response (88% versus 38% in Study RM-NTZ-98-002 and 56% versus 23% in Study RM02-3007) and parasitological response (75% versus 20% in Study RM-NTZ-98-002 and 52% versus 14% in Study RM02-3007). However, sensitivity

analyses and subgroup (based on age and gender)/special (*C. Parvum* infection only) population analyses did not, consistently, show statistically significant results.

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*,

Another double-blind, placebo-controlled study was conducted in hospitalized, severely malnourished children of age 1-11 years with acquired immune deficiency syndrome (AIDS) in Zambia. In this study, a three-day course of nitazoxanide suspension (100 mg BID in children ages 12-47 months, 200 mg BID in children ages 4 through 11 years) did not produce clinical or parasitological response rates or mortality rates that were significantly different from the placebo control.

Giardia Lamblia

Adult Studies

Adult HIV negative population with *Giardia Lamblia* was studied in only one placebo controlled study, conducted at a single center in Egypt. This study enrolled patients with either giardiasis or amoebiasis or both. The sample sizes were very small and hence the results of this study, although statistically significant, could not be generalized. Also, kappa coefficient for the NTZ arm in the group of patients with giardiasis was negative (-0.283), compromising the meaningfulness of the parasitological endpoint.

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Giardia Lamblia*

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies

The population of HIV negative children (age 2-11years) with *Giardia Lamblia* infection was studied in only one active controlled study, RM-NTZ-99-010, conducted at a single center in Peru, where metronidazole was used as an active control. In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole) , but not in terms of the parasitological response rates.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lamblia,* but not :

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